5

Detecting and Monitoring Harmful Agents

This chapter assesses current and emerging technologies and equipment for detecting the presence of harmful agents and monitoring changes in their concentration over space and time. The chapter describes DoD's current and planned techniques for (1) point and area sampling, (2) local and remote detecting, and (3) real-time and delayed analyses. More detailed descriptions of technologies and equipment can be found in Appendices D and E. The focus in this chapter is on the capabilities of technologies for detecting and monitoring agents at low concentrations.

Three key questions provide a framework for assessing detection and monitoring technologies:

1. Are current technologies for sampling and detecting harmful agents capable of answering questions on both short-term threats and the long-term health of deployed forces?
2. Will the technologies under development for sampling and detecting harmful agents be capable of answering questions on both short-term threats and the long-term health of deployed forces? (Until recently [post-Desert Storm], the requirements for chemical and biological detection systems were related only to acute exposures likely to affect a unit's ability to fight.)
3. What actions can DoD take to foster the development of and better use of sampling and detection technologies to protect the health of deployed forces?

The following criteria are used to evaluate individual technologies: reliability; sensitivity; selectivity (i.e., discrimination between the target substance and similar substances); speed; portability; and cost.

Measurements of concentrations involve physical and/or chemical techniques, such as mass spectrometry, light scattering, and enzyme interaction. The equipment includes one or more measurement technologies in a system for sampling, separating, detecting, and monitoring CB agent concentrations in air, soil, water, and food. The equipment often includes devices to record, store, transfer, and analyze data.

In evaluating technologies and equipment, a few overarching issues can be helpful. Table 5-1 shows the information needs and timing that detection/monitoring equipment must support before, during, and after a deployment. The portfolio of technologies and equipment being developed for deployments (along with doctrine for their use) should provide information that addresses these needs. The elements in Table 5-1 should be applied systematically to each class of agent (chemical warfare agents [nerve agents, blister agents, choking agents, etc.], industrial chemicals, and biological warfare agents).

Before deployment, harmful agents in the intended theater of deployment should be detected and monitored for intelligence purposes and for planning exposure assessments. During a deployment, real-time detection of harmful agents will be required to ensure that mission objectives are met and for continued monitoring. The information can be archived and used to determine low levels of chemical concentrations for dose reconstruction and long-term health risk assessments. Biological samples could also be collected for studies of postdeployment health effects.

In the sections that follow, technologies and equipment for detecting and monitoring chemical agents and technologies for recording and evaluating collected data are described. A matrix is presented showing, for each detector system (and for each chemical contaminant the system senses), the range at which contaminants are detected, the detection limit at maximum range, and the reliability of identification and quantification. Equipment for detecting and monitoring biological agents are then described. The chapter ends with descriptions of procedures and systems for recording and evaluating information.

DETECTING AND MONITORING CHEMICAL AGENTS

A wide variety of measurement equipment is available to DoD. Testing kits, detectors, and monitors of varying sensitivity (lowest level detectable) and specificity (ability to distinguish the target substance from similar substances) have been developed and/or used by the armed forces to identify concentrations of harmful agents. In addition, DoD,

TABLE 5-1 Information Needs and Timing for Measuring Short-Term Threats and Long-Term Health Risks

Information Needed	Before Deployment	During Deployment	After Deployment
Short-term threat	Intelligence and planning	Real-time measurements	Retrospective assessments
	Enemy CB capabilities	Contaminated areas	
	Means of delivery	Performance-degrading concentrations	
	Agents available	CB agent concentrations	
	Enemy troop CB protection	Location of enemy	
	Enemy CB doctrine	CB means of delivery	
	Prior CB use by enemy	Industrial sites with large stores of CB agents and TICs	
	Endemic CB threats in the region	Use of protective clothing	
	Large stores of toxic chemicals		
	Threshold concentration/time factors for any CB agents likely to cause short-term casualties		
Long-term health risk	Baseline data on exposures prior to deployment	Data that can be used to support health studies	Data on post-deployment exposures
	Susceptibility of troops to CB agents	Data on chemical concentrations and locations of these concentrations	Possible low-level exposure during deployment
	Threshold concentration/time factors for any CB agents likely to cause long-term health risks	Troop location and time histories	
		Use of protective clothing	

other federal agencies (e.g., EPA), and the private sector continue to develop technologies and equipment for detecting and monitoring concentrations of TICs in multiple environmental media.

Measuring Chemical Concentrations

Measuring the concentration of a chemical substance can be visualized as a three-step process (NRC, 1991b). First, the medium (air, soil, water, or food) containing the chemical substance is sampled. Next, the chemical substance of interest must be separated from or otherwise distinguished from other chemical species that are present. Third, the chemical is identified. In actual practice, these steps often overlap to varying degrees (see Figure 5-1). An example of a procedure with no overlap is the detection of aerosol-bound PAH compounds. First, airborne particles containing PAHs are sampled and collected on a filter. Next, the PAH compounds are separated from the particles and then separated as individual compounds by chromatography or a similar process. Finally, the individual PAH compounds are detected by fluorimetry or a similar process. Other measurement processes combine detection with separation. For example, gas chromatography with flame ionization includes separation (gas chromatography) and detection (flame ionization) in one step. Many remote or point measurement devices that use infrared beams combine sampling and detection and use software analysis to carry out the separation step. In some measurement methods, a single device does

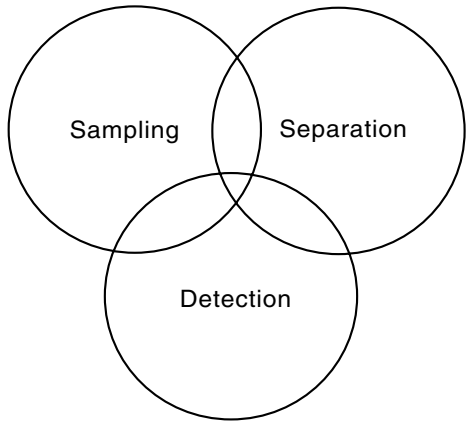

FIGURE 5-1 The three steps for measuring chemical concentrations in an environmental medium (air, water, soil, or food). Source: NRC, 1991b.

the sampling, separation, and detection. For example, a surface acoustic wave (SAW) detector draws in a sample, separates it on a membrane, and detects the agent with a single device.

Sampling

Sampling is the process of collecting the environmental or biological medium likely to contain the harmful agent. The sampling process can be active or passive; remote, stand-off, or local; mobile or stationary; personal or area. In addition, samples can be environmental or biological (e.g., breath, blood, urine, or hair).

Active and Passive Sampling

Chemicals dispersed in air as vapors or aerosols can be sampled actively or passively. (Vapor-phase chemicals are volatile chemicals found as gases in air. Aerosol-phase chemicals are either dispersed in air as droplets or are bound to particles). Active sampling requires that a person or automatic device direct and carry out the sampling. Passive sampling requires a minimum of equipment and a minimum of operator intervention. For example, airborne chemicals can be sampled actively using a pump to pull contaminants through a collection device. In contrast, passive sampling of airborne contaminants relies on diffusion to deliver airborne contaminants to the collection medium. The major advantage of passive sampling is that it does not require elaborate equipment and/or a number of well trained operators. The major disadvantage of passive sampling is the typically long time required to collect sufficient material for analysis. Passive sampling also tends to be less accurate than active sampling.

Remote, Stand-off, and Local (Point) Sampling

Remote sampling is done by equipment located at the point of interest but operated from a remote location. Stand-off sampling involves both the equipment and the operator being away from the location of interest. Local (or point) sampling is done by equipment and an operator at the location of interest. The advantage of the stand-off and remote approaches is that they provide advanced warnings by detecting agent concentrations before troops have any contact with the contaminated environmental medium. Remote and stand-off sensing of contamination can be conducted at various levels of spatial resolution using current military techniques and equipment, sometimes directed by intelligence information. Even though remote and stand-off sampling

are typically less accurate than local sampling, they are the sampling strategies of choice for protecting troops from potentially lethal clouds of agents. However, local sampling should be used for assessing low-level exposures because it provides more accurate measurements.

Mobile and Stationary Sampling

Mobile devices can provide samples of environmental media over a wide area that can be integrated to measure potential exposure. Mobile sampling increases the likelihood of finding local "hot spots." However, because mobile samplers must be light and portable, they are often not as accurate as stationary samplers.

Personal Sampling and Area Sampling

Area sampling of the air over a troop operation provides a measure of potential human exposure. However, personal sampling of the air in the breathing zone of an individual can provide a much better measure of exposure. The breathing zone is typically defined as the space within about one foot (30 cm) of the nose or mouth. For personal sampling, a small device is typically mounted on clothing that covers the chest. Measures of concentrations in the breathing zone are generally considerably higher when measured by personal sampling than when measured by area sampling, especially if the individual is engaged in activities that release or resuspend chemicals from soil in the area or from accumulated contamination on clothing.

Biological Sampling of Potentially Exposed Personnel

Personal badges and monitors can provide sufficient information to warn of certain gases and aerosols that could produce acute responses. However, for agents that can penetrate the skin after dermal exposure, or for some agents that are cumulative and produce delayed effects, biological monitoring of blood, urine, or hair can be analyzed for the presence of the agent metabolites, enzymes, and adducts in endogenous proteins or DNA. The utility of biological monitoring depends largely on knowing which metabolites are relevant. Most, if not all of these analytes, are likely to vary greatly in biological concentrations, and analyses can be quite expensive (Zhitkovich and Costa, 1998). Biological sampling and exposure assessments for deployed forces are discussed in detail by Lippmann (in press).

Sampling for Separation and Detection Technologies

Sample collection requirements vary greatly for different technologies. For example, active samplers linked to a gas chromatography/mass spectrometry system use small pumps to draw air through a collection medium, such as a filter or a vapor trap. Some detection devices require only a small amount of agent, others require much larger amounts. For some separation and detection technologies, the samples must be carefully stored and treated with a solvent before analysis.

Separating and Detecting Chemical Agents

Separation and detection technologies make use of the attributes of chemicals that distinguish them from other chemical compounds and make them detectable. These attributes include the mass-to-charge ratio of the molecule or atom; absorption and scattering of electromagnetic energy (particularly in the infrared to microwave region); chemical reactions that cause color changes; reactions with enzymes; physical characteristics that allow separation processes; electrochemical properties; and reactivity that causes unique emissions, such as chemiluminescence. Many detection technologies (e.g., mass spectometry) are based on some form of spectrometry, the use of the absorption, emission, or scattering of electromagnetic radiation by atoms, molecules, or ions to detect target substances qualitatively or quantitatively. A sensor is a device that produces a measurable response to a change in a physical condition (e.g., temperature or thermal conductivity), chemical concentration, or electronic charge. In Appendix D of this report, a number of technologies for detecting vapor-phase and aerosol-phase chemical agents, as well as chemicals in other media (e.g., water, soil, or food), are described.

Detecting and Monitoring Vapor-Phase Chemicals

The threats posed by many chemical warfare agents and TICs are most significant in the vapor phase. Analyses of samples of vapor-phase concentrations can reveal not only which agents are in the air but can also signal the presence of these agents in other media. Because the presence of vapor-phase chemicals is often transient, they must be detected quickly and accurately. Technologies that can detect chemical warfare agents in air, water, and food can, for the most part, be adapted to also detect industrial chemicals and other harmful chemicals likely to be found in the deployment environment.

Many toxic chemicals partition between the vapor phase and the condensed phase (including condensing onto the surface of airborne

particles), which can affect the health consequences of exposure to these chemicals. Thus, ideally, the amount of agent in the aerosol and vapor phase should be detected independently. Samples must be taken carefully to ensure that the procedure does not alter the distribution between the vapor and condensed phase.

A large number of technologies are available for detecting vapor-phase chemicals in the atmosphere, including color-change technologies, ion mass and mobility spectrometers, technologies based on infrared absorption and emission spectroscopy, chromatography, optical emission/absorption methods, physical- and chemical-process-based sensors, and enzyme methods.

Point (Proximate) Detection of Vapor-Phase Chemicals

Technologies capable of local detection of airborne chemicals are infrared spectroscopy methods. These include Fourier transform infrared (FTIR) spectroscopy and tunable infrared laser absorption spectroscopy, mass spectrometry, ion mobility spectrometry (IMS), enzyme methods, and phosphorous chemiluminescence detection (PCD). Each of these methods has advantages and limitations. Although FTIR is a mature technology, it requires a trade-off between speed and sensitivity. Mass spectrometry, which uses chemical ionization and quadrupole ion trap technology, is likely to outperform other technologies, but portability and speed can be problems. IMS has not demonstrated a level of performance that would justify its selection over other technologies. Enzyme immunoassays will never be fast and are likely to remain finicky to use but are as specific as any technology available. In laboratory studies, PCD has demonstrated the necessary speed (as little as one second response time), the necessary sensitivity, and no problems from interference. The response time will be longer if a gas chromatography step is required, which is likely in many situations. PCD is not likely to be included in hand-held or portable devices in the near future, however. Immunoassays can probably not be developed for all agents of interest because of variations in immunogenic properties among different agents. As a localized air-sampling technique, microwave spectroscopy appears to offer unambiguous chemical identification in real time without pretreatment. However, portability is a problem, and this technique does not work for medium or large molecules.

SAW is a promising technology, but it has not been tested in a wide range of field conditions, and sensitivity/specificity trade-offs are still a significant problem. SAW could provide a rapid, portable technology for personal monitoring but has the disadvantage of requiring that each agent

have a specific SAW coating on the surfaces where the acoustic detection occurs (DoD, 1997b). The SAW device will be difficult to adapt for the detection of TICs and other harmful chemicals because the device operates on the basis of target chemicals dissolving into the SAW's surface coatings. Because the span of solubility values is limited and not narrow valued, the number of target chemicals has to be restricted accordingly; interferences can compound the problem. The SAW device can detect and identify a wide range of chemical agents with only six different coatings. However, more coatings may be needed to achieve higher degrees of specificity for large target populations, such as TICs. If new agents respond to existing coatings, it will be fairly simple to change the detection software to recognize them. If not, new coatings will have to be developed.

Stand-off Detection of Vapor-Phase Chemicals

Currently, only FTIR and light detection and ranging (lidar) can be used for stand-off detection of vapor-phase chemicals (Stedman, 1999). FTIR provides passive detection, but it cannot detect all chemicals of interest. FTIR relies on spectral pattern recognition software to translate individual species concentrations out of complex multicomponent spectra. Thus, an important issue for detecting and monitoring TICs is that the equipment and software be properly calibrated for detecting specific chemical agents. In addition, operators must be trained to monitor chemicals other than chemical warfare agents. Calibration and training should be done before deployment. Like many other detection technologies, the specificity and sensitivity of lidar depend on proper calibration. Lidar is considered an active detection system.

Microwave spectroscopy has been considered but not yet demonstrated as a stand-off technique. One problem with microwave spectroscopy is extracting detailed information from pressure-broadened spectral signatures. It may also be difficult to separate the detection signal from microwave "noise" in the deployment arena.

Stand-off technologies, such as FTIR, have been used by EPA and private sector organizations to monitor air emissions. FTIR has the capability of measuring more than 100 of the 189 HAPs listed in Title III of the Clean Air Act. However, detecting multiple agents requires spectral-recognition software that can translate mixture spectra into component concentrations. This could limit the use of FTIR for complex mixtures of pollutants in low concentrations. When the Clean Air Act amendments were passed in 1990, measurement methods had only been developed for 40 HAPs.

Problems with Pollutant Interference

The problem with all vapor-detection technologies is that they must be able to distinguish one pollutant from another in a complex chemical environment. The problem is especially difficult for stand-off detectors, which work best when they can be calibrated to environmental conditions and types of chemicals. In most deployments, however, calibrating the equipment for the local conditions will be impractical, if not impossible. Because the specific target chemicals may not be well known, it will be difficult to calibrate detection devices for the hundreds of chemicals that could pose a threat to the deployment force.

Selectivity has also been a serious problem for most current local (point) detection equipment and all of the stand-off detection equipment. Selectivity will be an important capability of emerging technologies.

Aerosol-Phase Detection

Many harmful chemical agents, including chemical warfare agents and TICs, are dispersed in the atmosphere as aerosols or attached to atmospheric aerosols. Important characteristics of particles include size distribution, internal versus external mixing, and differences between the size distribution and composition of toxic particles and ambient particles. Identifying harmful agent particles requires defining the attributes of target particles, such as particle mass, particle number, and organic carbon content.

Detecting aerosol-phase chemicals requires either collecting and analyzing aerosol particles or using particle spectroscopy (i.e., infrared or lidar). Scientists are working to develop portable advanced instruments that can measure the size, mass, and chemical composition of individual airborne particles in real time. Currently, aerosol mass spectrometry is used to characterize atmospheric aerosols. However, many emerging technologies have the potential for assessing the size distribution and chemical composition of atmospheric aerosols.

Current Methods

Aerosol mass spectrometers, which measure particle size, are currently used to characterize atmospheric aerosols. Mass spectrometers work in two stages: particle sizing followed by mass spectroscopy (Gard et al., 1997; Green et al., 1998; Johnston, 1999; Noble and Prather, 1996; U.S. Army SBCCOM, 1998). Particle sizing is achieved by different methods. One approach is to measure particle time of flight by timing light-scattering signals from different laser-beam probes. When

the difference in mass-to-charge ratio of ionized aerosol particles is used to characterize chemical composition, mass spectroscopy is used after the aerosol particles are vaporized. Composition attributes that can be derived from the mass spectra include the dependence of composition on particle size, comparison of surface composition to total composition of the particle and (in some cases) composition of the organic molecule.

The goal of aerosol mass spectrometry is to provide on-line, real-time chemical analysis of individual aerosol particles, which are characterized in terms of bulk composition, surface composition, organic chemical species, and inorganic chemical species. An on-line system minimizes sampling artifacts caused by condensation, evaporation, and/or chemical transformation. A real-time system provides high temporal resolution and can monitor rapid changes in particle composition.

Only a few adequate on-line techniques are available for detecting and characterizing small aerosol particles. Conventional methods involve isolating particles on filters followed by analysis in the laboratory. The isolation processes often disturb the aerosol and thus render the data questionable because particles can evaporate or react before analysis. Aerosol spectrometers use lasers or hot surfaces to volatilize aerosols. Newer spectrometers that use gentler vaporization strategies will probably overcome this problem. An example of an emerging technology based on aerosol spectrometry is aerosol time-of-flight spectrometry (ATOFMS), which provides the size and chemical composition of individual aerosol particles in real time (Noble and Prather, 1996). With sufficient development funding, ATOFMS could be made field portable in the next decade. It is not likely, however, that it could be made small enough to be used by an individual soldier.

Criteria for assessing the performance of aerosol-agent detection devices include reliability, sensitivity, selectivity, speed, portability, and data archiving. Current on-line methods for assessing aerosol-phase chemicals are becoming more reliable, and field measurements are now routinely performed by aerosol mass spectrometry. One concern about the reliability of this technology is whether the laser/particle beam alignment will remain stable under the extreme conditions of a deployment. The sensitivity of these devices is improving. Historically, chemical concentrations were determined empirically from particle characteristics; now, the chemical composition of individual particles can be better analyzed, and particles can be quantitatively grouped by composition and counted. In addition, if organic chemicals on particles are not badly fragmented from volatilization, individual chemical concentrations can be determined to the parts-per-thousand level for individual particles.

Particles can currently be quantitatively grouped by composition only if internal mixing does not occur. Distinguishing among organic species

remains difficult because water and other contaminants in the air may alter the observed spectra. Up to 10 particles per second can now be routinely analyzed under favorable conditions. New systems using hot surface vaporization instead of laser vaporization can size and then chemically assess thousands of particles per second (Jayne et al., 1998).

Portability remains a problem for current systems. Field aerosol mass spectrometers using laser vaporization typically require more than 30 amps of continuous power and weigh a few hundred pounds. Smaller versions are under development. Devices that use surface vaporization can be smaller and require less power. The use of an IMS may reduce the power requirements to 5 amps and the weight to 10 pounds.

Current mass spectrometer systems are compatible with archiving real-time data. Single-particle mass spectra are digitally recorded and can be analyzed automatically.

Emerging and Future Developments

Technological improvements are likely to increase the reliability, sensitivity, selectivity, speed, and portability of devices for detecting aerosol-phase agents. Enhancements to basic methods of mass spectrometry will be one important source of improvements. SAW technologies have the potential for detecting aerosol-phase chemicals and are being investigated although the coating solubility problem will have to be overcome. Lidar is being considered for stand-off assessments of particles and has the potential for detecting aerosol-phase chemicals. Lidar would require the development of absorption spectra for particles and aerosol-phase chemicals.

Detecting Chemicals in Water, Food, and Soil

Some of the chemical detection technologies used for detecting vapor-phase chemicals can also be used for detecting chemicals in water, food, and soil. Chemiluminescence can take place in either the solution or vapor phase and thus can be used for detecting chemicals in water. Determining the presence of chemical agents in food and water is most often performed with the assistance of a gas chromatograph/mass spectrometer following an extraction step. Liquid chromatography, which is used to separate analytes in a solution, works with both metal ions and organic compounds. The mobile phase of the separation column is a solvent, and the stationary phase is a liquid on a solid support, a solid, or an ion-exchange resin. Most agents in food and soil cannot be detected directly or in real time but require a solvent-extraction step.

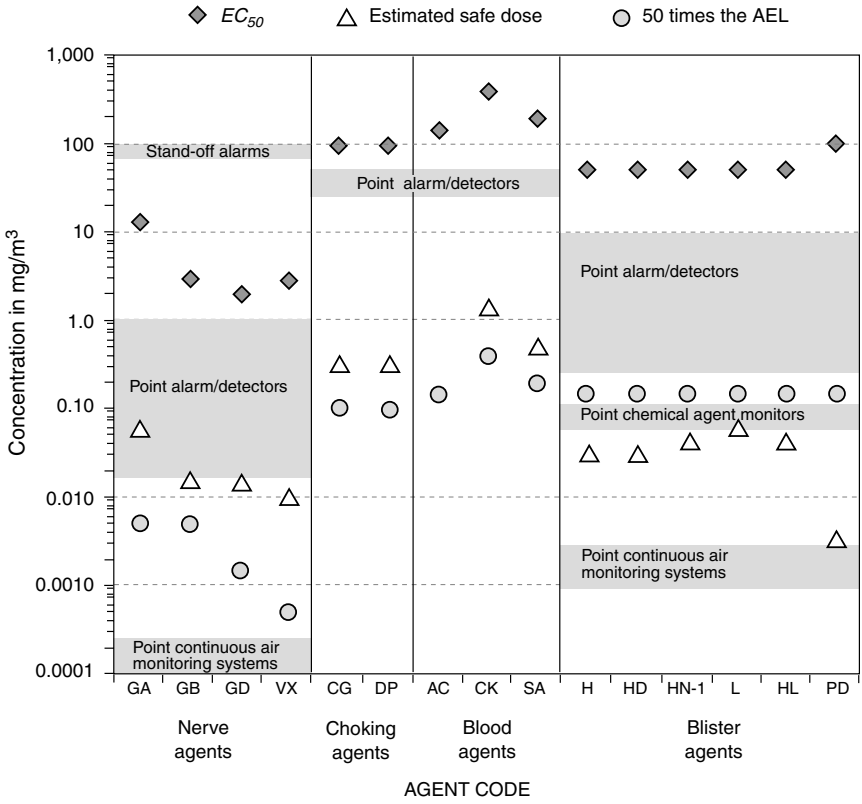

FIGURE 5-2 Detection sensitivities for detection equipment compared to the EC_{50} (the 30-minute average air concentration that would result in the LCt_{50}), DoD's estimated safe concentration, and the AEL.

Summary Evaluation of Chemical Detection Technologies

DoD's stated strategy for chemical detection is to use a suite of complementary technologies to ensure enough warning time for contamination avoidance (JCS, 1996). Figure 5-2 provides a summary review of the chemical detection/monitoring technologies and other devices discussed in this chapter. A comparison of the lethal levels and DoD's "safe" concentrations to device sensitivities shows that current technologies do provide a margin of safety from lethal exposures. However, only complex, nonportable systems have sufficient sensitivity to detect the AELs.

Detecting concentrations near the AEL will be a measure of the value of emerging equipment for detecting low-level exposures. For example, the joint chemical agent detector (JCAD) will be more selective, more sensitive, and more portable than current equipment but may not be sensitive enough to fully address low-level exposures. Sensitivity at AEL-level concentrations has not been demonstrated in field tests for any emerging technology.

Current equipment is designed primarily to detect nerve and blister agents. Choking, blood, riot-control, and psychochemical agents, as well as biological toxins and TICs, are not high priorities in the design specifications of available equipment. The only devices explicitly capable of detecting these agents are large gas chromatography systems. The priorities for future equipment continue to focus on nerve and blister agents. The speed of detection is likely to continue to increase for all detection technologies.

DETECTING AND MONITORING BIOLOGICAL AGENTS

At present, the capability of detecting biological agents is limited. However, DoD has identified the need for local (point) and stand-off, real-time biological agent detection and has given the development of this capability a high priority for the near future. The following discussion provides a review of several existing and emerging technologies and tools for detecting biological agents during deployments. More detailed descriptions of these systems are provided in Appendix E. This appendix includes a review of each system's local and stand-off sampling capability, personal sampling capability, use or calibration with biomarkers, and use of surrogate samples.

Measuring Biological Organisms

Numerous methodologies are currently available for detecting biological material collected from environmental samples. No one analytical method is likely to support all requirements for all situations, however, so selection criteria will help in the selection of an appropriate analysis method. Table 5-2 is a summary of major criteria and supporting considerations for detection and monitoring devices at fixed sites as well as mobile facilities.

Sample Matrix

An analysis of environmental samples for microbial contaminants encompasses a variety of matrices (i.e., substances that contain biological organisms), including air, water, surfaces, and food products. Collection

TABLE 5-2 Criteria for Selecting Analytical Methods for Detecting Biological Contaminants

Criterion	Considerations
Matrix sampled	Collection medium Temporal and spatial variability Interference from indigenous microbial populations and background constituents
Type of information needed	Qualitative/quantitative data Level of specificity Level of sensitivity (detection limits)
Integrity of sample	Storage prior to analysis Archiving capability
Analysis timetable	Turnaround time/speed of analysis Continuous/real-time versus batch analysis Capability of multiple analyses
Physical design	Reliability Portability Resistance to countermeasures Nonvolatile memory
Data interpretation	Accuracy Precision Reproducibility

strategies for each matrix could involve an assortment of sample media. The analysis method must be matched to the environmental matrix and to the collection medium. In addition, the detection of microbial contaminants is confounded by the ubiquitous presence of microorganisms and their by-products in the environment. The presence, composition, and concentration of microorganisms are heterogeneous and highly variable. Except in unique indoor situations (e.g., clean rooms associated with pharmaceutical facilities), the concentration and composition of microbial populations is highly variable over time and space, often fluctuating by several orders of magnitude. Abiotic constituents in the environment may also interfere with the detection of microbial contaminants.

Type of Information Needed

Qualitative data indicate the presence or absence of biological contaminants at a predetermined threshold. Quantitative data would

provide a numerical measure of biological contaminant(s). Specificity refers to the required level of discrimination among biological agents.

The genus level of microbial taxonomy is further divided into species, subspecies, and strain classifications. For example, the genus *Bacillus* contains numerous species, but the biological contaminant of interest may be the *Bacillus anthracis* present in a background of indigenous, nonpathogenic *Bacillus* species. Sensitivity (the range of measurements achievable) is often dictated by the physical limitations of the analysis methodology. The lowest possible detection limit will minimize dilution effects of the dispersion of the microbial contaminant in the environment and in sample collection. Although zero presence of an agent may be desirable, acceptable sensitivity levels are determined by the dose of the microbial contaminant that causes adverse effects in the exposed population.

Sample Integrity

Collection and preservation requirements are critical to the detection of biological contamination, as the integrity of the microbial populations within the sample is likely to change over time. Rapid processing/analysis at the time of collection can minimize problems with the preanalysis integrity of samples. Postanalysis archiving is a problem with all current methods.

Analysis Timetable

The speed of analysis, or the number of samples that can be analyzed in a given time, includes considerations of the analysis time per sample and the number of multiple samples that can be analyzed simultaneously per instrument.

Physical Design

Reliability, portability, resistance to countermeasures, and nonvolatile memory are engineering design goals for analytical technologies. Advances in miniaturization and microcircuitry have reduced once cumbersome methods to field-portable units for use by ground troops and mobile facilities. Communication links through digital satellite-based transmission can provide rapid data distribution for remote interpretation and archiving.

Data Interpretation

Accuracy is defined as the level of agreement between measurements and an accepted reference standard. Precision is a measure of agreement among individual measurements of the same property under the same or similar conditions. The reproducibility of data is determined by analyses performed on replicate aliquots of a single sample. Although these considerations are critical to assessing the capabilities of a biological detection technology, they are often not reported in the literature.

Emerging and Traditional Detection Technologies

Traditionally, the detection of microorganisms has been based on microscopy, culturing techniques, biochemical assays, and immunoassays. Microscopy is used to detect microbial populations in a given sample without regard to the physiological state of the organism; both viable and nonviable organisms can be detected. Because classical microscopy relies on the recognition of morphology (size and shape), limitations of this technique include lack of specificity and low sensitivity. Staining with fluorescent-labeled antibodies can result in the detection of target organisms, but the lower detection limits are generally greater than 10^4 cells/ml of liquid collection medium (ideal detection strategies would detect one cell in a sample). The detection of submicroscopic viruses requires specialized instruments, such as a transmission electron microscope.

Culture-based assays are limited to the detection of organisms that proliferate under the growth conditions of the analysis design. A successful culture depends on nutritional and environmental factors, the physiological state of the organism, and the presence of interfering substances. Stresses induced during dispersal, transport, and collection can increase the difficulty of detecting organisms. Analysis time is dependent on the organism, the growth medium, and the incubation temperature. However, 18 hours is generally required for the formation of a bacterial colony. Detection limits are highly variable depending on the application of the sample to the growth medium.

Biochemical-based and immunological-based analyses have improved the identification and enumeration of specific microbial contaminants in environmental samples. Generally, biochemical assays rely on a substrate and computer-assisted analysis. Immunoassays center on specific antigen-antibody recognition. When used sequentially with culture techniques, these immunoassays afford increased specificity. However, the analysis time is prolonged. Advances in nonculture-based immunoassay are expected to result in improved specificity and sensitivity.

Emerging Technologies

Improved detection and identification of microorganisms can be achieved with advanced biotechnology-based methodologies, including polymerase chain reaction (PCR) amplification; microchips; molecular beacons; electrochemiluminescence; biosensors; mass spectrometry; and flow cytometry. Brief summaries of these technologies are provided below. More detailed descriptions can be found in Appendix E.

PCR involves the use of unique primers to amplify DNA products. Reverse transcriptase PCR is used to detect ribonucleic acid (RNA) by generating a DNA copy of the nucleic acids in a single-stranded RNA. Detection limits are affected by the physical condition and concentration of the target nucleic acids. The presence and concentrations of background biotic and abiotic material may require that samples be pretreated to minimize interference in the sample matrix. Combining PCR with immunological techniques has resulted in a rapid and efficient solution-phase hybridization of labeled targets and biotinylated capture probes.[1] Results have been reported in two hours with a detection limit of 10 targets, which is relatively good for biological agents. Other methods may take from hours to days. Further information on advanced PCR analysis methods can be found in Alvarez et al. (1995), Beyer et al. (1995), Buttner et al. (1997), Friedman and Meldrum (1998), Garner et al. (1993), Herman et al. (1997), Kai et al. (1997), Kuske et al. (1998), Lindqvist et al. (1997), Lopez et al. (1996), Rigler et al. (1998), Sandery et al. (1996), Sawata et al. (1997), Suzuki et al. (1992), and Wu et al. (1997).

Integrating microchip technology and PCR has improved detection. A microchip-PCR array with 10 silicon reaction chambers, thin-film heaters, and solid-state optics can provide real-time monitoring with low power requirements and no moving parts. For in-depth information on microchip technology, the reader is referred to Belgrader et al. (1998), Ibrahim et al. (1998), Northrup et al. (1998), Waters et al. (1998), Wilding et al. (1998), and Yershov et al. (1996).

Nucleic acid probes that spontaneously undergo a fluorogenic conformational change when they hybridize with target fluorescent probes are called "molecular beacons." These beacons are specific, that is, they fluoresce only in the presence of a complementary target. Reactions are

[1] Biotinylated capture probes are constructed using biotin conjugated to a monoclonal antibody labeled with a fluorescein or rhodamine dye, enzyme, or isotope conjugated with avidin. When the avidin-labeled monoclonal antibody-biotin structure interacts with the targeted microorganisms, the reaction is detected with immunoassay, ELISA, or radioimmunoassay, depending on the label.

carried out in a sealed tube minimizing manipulation (Tyagi and Kramer, 1996).

Electrochemiluminescence technology integrated with equilibrium immunoassay provides detection ranges from 2.5 ng/ml to 2000 ng/ml with an accuracy and precision of less than or equal to 15 percent for human protein sequence and 0.5 ng/ml to 200 ng/ml for mouse protein sequence (Grimshaw et al., 1997).

Biosensors involving immunoassays in conjunction with a flexural plate wave transducer membrane have been used for the detection of bacteria. Current detection limits are relatively high (3.0×10^5 to 6.2×10^7 cells/ml) (e.g., Harteveld et al., 1997; Pyun et al., 1998).

Gas chromatography-ion trap tandem mass spectrometry and conventional quadrupole gas chromatography/mass spectrometry have been used to detect 3-hydroxy fatty acids (e.g., endotoxin; bacterial lipopolysaccharide in gram-negative cells), muramic acids (e.g., peptidoglycan in gram-positive and gram-negative bacterial cells), and ergosterol (fungal biomass) as indicators of the presence of microbial contamination. For discussions of advances in mass spectrometry, the reader is referred to Kaufmann (1995), Koster et al. (1996), Krahmer et al. (1998), and Larsson and Saraf (1997).

Flow cytometry utilizes simultaneous measurements of light scatter to determine cell size and structure. Fluorescence increases the capabilities to include quantitation of cellular components, antigen detection, and estimations of cell physiology (see, for example, Davey and Kell, 1997; Fouchet et al., 1993; Lange et al., 1997; and Perez et al., 1998; Seo et al., 1998). Instrumentation permits the measurement of 500 to 5,000 objects per second with the results displayed in bivariate histograms. Even though the combination of flow cytometry and fluorescent *in situ* hybridization has increased detection by two orders of magnitude over culture-based assays, detection rates below 10^2 cells are beyond the capabilities of currently available detectors. Immunomagnetic separation with fluorescent antibody-labeled beads and flow cytometry is also being used (Seo et al., 1998).

Fielded Equipment for Biological Agents

Current biological detection equipment is not as mature as chemical detection systems in terms of reliability, sensitivity, selectivity, speed, and portability. Rapid, remote detection of biological agents is based on analysis and the collection of aerosols. Point samples of soil or of aerosol currently must undergo microscopy and culture methods for a definitive identification and count of biological organisms. Some currently available detection equipment is listed below (DoD, 1999a; U.S. Army SBCCOM, 1998):

- the biological integrated detection system, a collection of components used to provide mobile detection capability (Berry, 1998)
- the interim biological agent detector, a point detection system used to detect background changes indicative of human-made biological warfare agents
- the XM94 long-range biological stand-off detection system, which provides long-range, large-area aerosol cloud detection and ranging and tracking capability
- the FOX nuclear, biological, and chemical reconnaissance system, a lightly armored, wheeled vehicle that can collect samples for laboratory analysis but is not capable of detecting or identifying biological material.

Emerging Equipment

An effective defense against biological warfare agents will require real-time, preexposure detection, discrimination, and identification of the threat. To address this requirement, several agencies, including the Defense Advanced Research Projects Agency, are focusing on the development of robust, unattended, real-time (less than 1 minute), highly sensitive (2 to 10 particles), small (less than 5 pounds), low cost (less than $5,000/unit) detection systems. The detection of biological warfare agents on the battlefield in real time with a very low rate of false alarms is a crucial requirement. However, with the possible exception of upconverting phosphor-diode laser technology, no technology currently under development is expected to meet these needs in the next five years.

DATA COLLECTION, RECORDING, AND STORAGE

Detection and monitoring systems provide valuable information for personnel in the immediate area of the equipment, as well as for forces and support personnel in the wider theater of deployment. Some existing equipment and many developing technologies not only provide a warning alarm, but also record, store, and transmit information on levels of chemical agents. Information storage and retrieval are crucial to postdeployment assessments of exposures.

Warning and reporting are the critical links between CB detection and CB protection and medical support. In addition to detection and monitoring, commanders need accurate, timely information about the concentrations of harmful agents. Collecting, evaluating, reporting, and storing information are critical issues in contamination avoidance. Currently, collection and transmission of information on threats are managed

through conventional communications channels. However, DoD is pursuing the development of dedicated hardware and software to collect, transmit, integrate, and evaluate CB information. These systems will also provide information management and control functions. The multipurpose integrated chemical alarm (MICAD) and JWARN are systems designed to perform these functions. Another concept, the joint biological remote early warning system (JBREWS) is planned to be a "system of systems" that will integrate several other systems, as well as miniature detectors.

Multipurpose Integrated Chemical Alarm

MICAD is an emerging integrated nuclear, biological, and chemical detection, warning, and reporting system. It automates the gathering of NBC contamination data from fielded detectors and sensors and automatically gives alarms and transmits reports up the chain of command. MICAD is not a detector; it is a system that collects, stores, and transmits information received from an array of detection devices, such as the M22 automatic chemical agent detection alarm chemical detectors.

Joint Warning and Reporting Network (JWARN)

The JWARN is being designed to provide joint forces with a comprehensive analysis and response capability to minimize the effects of NBC attacks or accidents/incidents (DoD, 1997b, 1999a; U.S. Army SBCCOM, 1998). JWARN will provide the operational capability to use NBC warning technology that can collect, identify, analyze, and disseminate threat information. The new system, which will be compatible with and integrated with other joint service systems, will be located in command and control centers and used by NBC defense specialists and other designated personnel. It will transfer data automatically to and from the detector or sensor and provide commanders with analyzed data for decisions on disseminating warnings to the level of individual soldiers on the battlefield. It will provide data processing, plans and reports, and access to specific NBC information for optimal use of limited resources.

JWARN is a three-phase program. Phase I includes the procurement of analysis software, the development of detector protocols, and the development of an interim field capability. Phase II will provide the total JWARN capability by integrating detectors and additional NBC software modules into the services command, control, communications, computer, intelligence, surveillance, and reconnaissance (C4ISR) systems. Phase III will upgrade JWARN communications and software to work with the next generation of detectors.

System Goals

An important purpose of systems such as MICAD and JWARN is to increase the warning time by eliminating the manual and voice transmission of data and replacing it with automated transmissions. With increasing numbers of detectors in the deployment theater and increasing sensitivities, these systems will be useful for assessing both immediate threats and low-level exposures to CB agents and TICs. However, because of the large amount of information, screening and prioritizing will be necessary to keep from overwhelming commanders. Even with computer automation, decisions will have to be made about who collects CB information, when and how it is transmitted, how the information is archived, and how and when it is retrieved. Incorporating nonvolatile memory in the data management system will be another important goal of these systems.

MONITORING, SIMULATION, AND DECISION MAKING

The information obtained from detecting and monitoring devices will be very valuable both for anticipating and avoiding potential exposures and for determining the distribution of exposures in postdeployment health studies. Monitoring exposures for individuals requires tracking the time sequence of chemical concentrations in one or more media (air, soil, water, food, etc.) at a specific location. It also requires tracking the locations and activities of individuals to assess their level of interaction with the contaminated media.

Not all media, all locations, and all time periods can be monitored for all potentially harmful agents. Obtaining that information would probably require more troops and equipment than the deployment mission itself. Thus, assessments will have to be based on exposure information and extrapolated from a limited number of samples. Also, decisions about contaminant avoidance, the use of protective equipment, and the need for medical surveillance will have to be based on uncertain or incomplete information.

To reduce uncertainties, sampling strategies should maximize the amount of information that can be obtained from a limited number of detection devices, and computers should not only log and display the information but also make simulations on the levels of risk patterns of detected concentrations and weather conditions.

TESTING EQUIPMENT AND FIELD DEMONSTRATION

Testing equipment is an important aspect of each stage of the R&D process. Site visits and reviews of the technology development process during this study revealed that substantial testing and demonstration of new equipment has been done. Nevertheless, these tests are typically designed only to demonstrate that a technology can work. Many field tests are restricted to Dugway Proving Ground or White Sands Missile Range, the only places properly equipped for full-scale field tests. Independent scientific reviews at each stage of the development and testing process appear to have not been done, which could limit the quality and reliability of the final product.

The most important attributes of detection and monitoring systems for field use are reliability, sensitivity, selectivity, speed, portability, resistance to countermeasures, and nonvolatile memory. A definition of these functional attributes should include the following issues. Reliability should include operational reliability, informational reliability (integrity), and a failure mode (warning or no warning). Sensitivity refers to the detection limit of an analytic technique and is a relative concept. For harmful CB agents, the sensitivity of a detection or monitoring device varies with the concentration of the agent being detected or sampled. Most harmful agents have a threshold concentration at which the likelihood of health effects exceeds an acceptable value. A useful detection device for a harmful agent should be sensitive at concentrations that correspond to the thresholds of likely health effects. Selectivity should be assessed in terms of how comprehensive the device is (i.e., how many agents can be detected), the rejection of interference chemicals, and identification of multiple harmful chemicals from a large set of chemicals in the environment. Speed should relate not only to how quickly an agent can be detected but also how quickly the device can be made operational in the field. Portability should be specified in terms of person-portability or vehicle-portability. Resistance to countermeasures must be defined by how well the device performs in the presence of decoys or electronic jamming. Nonvolatile memory refers to the ability of a device to retain data that has been recorded in case of a power failure or other disturbance.

FINDINGS AND RECOMMENDATIONS

Finding. Overall, the capabilities of technologies and equipment either in use or under development are severely limited in their measurements of concentrations associated with long-term health risks. A significant reason for this problem is that no formal requirements have been established for detecting and monitoring low-level, long-term exposures. Until acceptable low-dose exposures are specified, performance goals for

low-dose detection technology cannot be established. Specifications would provide designers, developers, and operators of detection and monitoring equipment with goals for their research.

Recommendation. The Department of Defense should establish criteria for detecting and monitoring low-level exposures to chemical and biological warfare agents and toxic industrial chemicals. These criteria should specify three detection levels: (1) immediate, dangerous, and life-threatening hazards; (2) short-term hazards; and (3) long-term health risks.

Finding. Because different technologies have different strengths and weaknesses, no single technology should be relied on for detection. By using complementary and redundant technologies and sensor fusion techniques, which are commonly used in other areas of the military (e.g., air defense and antisubmarine warfare), the risk of false alarms could be reduced, and agents could be detected at lower limits.

Recommendation. At least two different but complementary technologies should be used, along with sensor fusion techniques, for the detection of a given type of agent. This combination could significantly reduce the number of false positives and false negatives.

Finding. Most of the equipment currently available, as well as most of the equipment under development, for sensing CB agents is designed for detection and warning only. Detection devices typically give off audible or visible signals when the concentration is above the sensitivity level of the device or above a preset value. These devices are valuable for protecting troops from immediate harm but do not provide the kind of monitoring needed to assess less-than-debilitating exposures or to assess exposures that might have delayed health effects.

Not enough attention has been given to archiving the measurements from different detectors. In some cases, archiving is not possible because of the nature of the device. Devices operated for "warning only" cannot be used in combination with systems like the multipurpose integrated chemical alarm and JWARN to determine the spatial and temporal trends in agent concentrations—essential information for determining the evolution of a threat or for confirming the absence of an agent.

Recommendation. The Department of Defense should develop a comprehensive plan for collecting and archiving data and samples based on a matrix of short-term threats and long-term health risks for situations before, during, and after deployment. This matrix could be used to prioritize the different types of information required.